

PHYTOCHEMICAL CONSTITUENTS AND HYPOGLYCEMIC EFFECT OF GYMNEMIC ACID EXTRACTS FROM BIG AND SMALL LEAF VARIETIES OF *Gymnema Sylvestre* R.Br

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ABSTRACT

Phytochemical and hypoglycemic effects of two varieties of *Gymnema sylvestre* were studied. The leaves of Big-leaf (MCL) and the small-leaf (ZMGL) varieties were grown in Uka Tarsadia University, Bardoli, India. Water, ethanol, methanol, petroleum ether, hexane and chloroform were used for phytochemical extraction from the dried leaves. Gymnemic acid was extracted and purified from the two varieties by Thin Layer Chromatography (TLC). Four groups of matured whisker rats (six per group) were induced with high-glucose level. Three groups were treated with standard drug glibenclamide (5mg/kg), gymnemic acid extracts from MCL and ZMGL respectively while the fourth group served as experimental control. Hypoglycemic activities of extracts were evaluated using animals' blood sample. Result showed water and methanol are best solvents for phytochemical extraction from the plant and the yield is higher in ZMGL than MCL. Also, gymnemic acid yield varied. From the results, hypoglycemic activities from the animals' blood revealed that treatments from both MCL and ZMGL are not significantly different from standard drug glibenclamide. The study concluded that water or methanol is appropriate solvent for phytochemical extraction from *G. sylvestre* leaves and extract from both lines of the plant could be utilized to reduce blood glucose level

Keywords: phytochemical extraction, extraction solvents, gymnemic acid, hypoglycemic effects

INTRODUCTION

In the recent decades, with increasing urbanization and changing lifestyle, prevalence of diabetes is increasing at alarming rate. Diabetes is a chronic disorder of carbohydrate metabolism which results in high blood sugar levels (Gunjan *et al.*, 2011). It is considered as one of the five leading cause of death in the world and it is estimated that diabetes would affect approximately 57 million people by the year 2025 (Grijesh *et al.*, 2009). Treatment and management of human diseases using herbs is not new though; many traditional and orthodox medicines in use are derivatives of medicinal plants (Joseph and Jini, 2010). World Health Organization (WHO) has listed 21,000 plants of medicinal important around the world. Among these, about 150 species are used commercially on a fairly large scale (Zohary *et al.* 2000). India is a major exporter (70%) of

crude drug extract and (30%) of finished products (ICMR, 2013) from herbs and medicinal plants.

Gymnema sylvestre (Retz) R. Br. is a slow growing, perennial, woody, climbing herb which belongs to Asclepiadaceae family. It grows in the tropical forests of Central and Southern India and can also be found in the tropical forests of Africa, Australia, Vietnam, Malaysia and Sri Lanka (Ye *et al.*, 2000). The leaves of the plant when chewed deactivates the sweet bud's ability to detect sweet or sugary taste for several hours. Among the local people of India, the leaves are routinely used for control of diabetes and it is well recognized in traditional medicine as a remedy for diabetes mellitus; hence, the leaves are being sold along with the aerial parts as Gurmarbuti in most herbal markets in the country (Mitra, *et al.*, 1995; Ankit, *et al.*, 2010).

G. sylvestre leaves contains several phytochemicals and gymnemic acids which is the most important constituent of the leaf extract have anti-diabetic, anti-sweetener and anti-inflammatory activities. (Liu *et al.*, 1992; Nakamura *et al.*, 2010). In addition, *G. Sylvester* leaf extract reduced high blood glucose in laboratory animals with potentials of treating diabetes in human and purified gymnemic acids are widely used as experimental reagents in taste physiology (Hellekant *et al.*, 1998; Kanetkar *et al.*, 2004). *G. sylvestre* has been reported to be safe in management of diabetes and short term uses of low doses may have unnoticeable side effects (Ogawa *et al.*, 2004).

Considering the foregoing and the fact that orthodox drugs for the management of hyperglycemia may not be within the reach of the rural economy less buoyant people, there is need to investigate phytochemical compositions of *G. sylvestre* with the view to extract a cheaper, safe but effective herbal products for the management of hyperglycemia. The present study therefore seeks to compare the phytochemical constituents and gymnemic acid yield of two common varieties of *G. sylvestre* and to investigate the effectiveness of (leaf extract) gymnemic acid hypoglycemia effects on whisker rats.

MATERIAL AND METHODS

The leaves of Big-leaf variety (MCL) and the Small-leaf variety (ZMGL from Research and Development Medicinal Plant Garden, Zandu Foundation for Health Care (ZFHC), Vapi, India) of *Gymnema sylvestre* grown in the Medicinal Plant Garden, College of Pharmacy, Uka Tarsadia University, Bardoli, Gujarat, India were collected and used for the study. Extracts from the two varieties were compared for amount of phytochemical constituents and gymnemic acid yield as well as their effectiveness in the management of hyperglycemia in laboratory animals.

Leaf extraction

The leaves were thoroughly washed with tap water and shade dried for 15 days. 20g of dried leaves and different solvents (water, ethanol, methanol, petroleum ether, hexane and chloroform) were used for extraction (Sofowora, 1993) to determine the most

effective extraction solvent for phytochemical screening and physiochemical analysis of the leaves extract.

Qualitative and quantitative determination of bioactive compounds in the leaf extracts

Qualitative determinations for the presence of bioactive compounds were carried out. Saponins, carbohydrate, flavonoids, glycosides, alkaloids, terpenoids and tanins presence was determined by methods described by Sofowora (1993) and Kokare (1999).

The amounts of Tannin, Saponin, and Alkaloid present in the leaves extract for each of the lines was determined using the method described by Obadoni and Ochuko, 2001, Phenol and Flavonoid quantification was done according to Yadav *et al.* (2011), carbohydrates determination as described by Hedge and Hofreiter (1962), while protein was estimated based on Bradford (1976) procedure. The DPPH free radical scavenging activity of the leaf extract was determined according to the procedure of Gazella *et al.* (2012) and the estimation was done using the relationship $\text{DPPH scavenging activity (\%)} = \{1 - (\text{Abs}_{517} \text{ sample} / \text{Abs}_{517} \text{ DPPH solution})\} \times 100$

Physiochemical analysis

Total ash, acid insoluble ash, water solubility, ether solubility and pH values of the leaf extracts for the two varieties of *G. sylvestre* were evaluated using the method of Khandelval (2008).

Extraction of Gymnemic acid

Different extraction methods were utilized for the extraction of Gymnemic acid from the powdered dried leaves of two varieties of *G. sylvestre* to compare the yield. Isolation of gymnemic acid was carried out according to Hooper (1987). Other methods were as described by Puratchimani and Jha (2004) and Gupta (2003). Methods for extraction of gymnemic acid and solvent system for thin layer chromatography with different Rf values (Table I).

Hypoglycemic effect of gymnemic acid on experimental animals

Healthy male whisker rats (250-300g) were kept on 12h light/dark cycle and had free access to food and water prior to laboratory experiment. The animals were acclimatized to

Table I. Methods for extraction of gymnemic acid and solvent system for TLC.

Method Ref:	Staining reagent	Solvent system	Reference Rf value
1. Hooper, 1987	0.5% Vanillin stain	Toluene: chloroform: methanol (5:8:3)	0.30
2. Puratchimani and Jha, 2004	0.5% Vanillin stain	Chloroform : methanol (9:1)	0.37
3. Puratchimani and Jha, 2004	Anisaldihyde	Chloroform : isopropanol : ammonia (2: 8: 2)	0.45
4. Gupta 2003	5% methanolic sulfuric acid	Chloroform : methanol : water (8.86 : 4.77: 1.36)	0.07, 0.13, 0.27, 0.36, 0.48, 0.56, 0.63, 0.75

the laboratory environment for an hour before the experiments. They were randomly distributed into four groups of 6 animals each. All experiments were conducted during the day (08.00-17.00h.). The procedures for testing the hypoglycemia effectiveness of the leaf extracts in comparison with standard drug of glibenclamide 5mg/kg on the whisker rats was according to (Cretti *et al.*, 2000). Blood samples were collected through retro-orbital plexus prior to commencement of treatment and at 30 minutes intervals up to a period of 150 minutes after glucose challenge. All the protocols were approved by the Institutional Animal Ethical Committee (IAEC), India.

Statistical analysis

The data obtained were subjected to the analysis of variance (ANOVA) using SPSS version 16.0 for Window Operating System and the effectiveness of the treatments was compared using Tukey's Multiple Comparison Test (TMCT). The level of significance difference between treatments was evaluated at the probability of $P < 0.05$.

RESULTS AND DISCUSSION

The qualitative analysis of crude samples from the two varieties of *G. Sylvestres* used for this study to determine presence of phytochemical compounds showed varied results for the presence or otherwise of phytochemical compounds with respect to different solvents of extraction as summarized (Table II). The result of preliminary screening of leaf extracts of *G. Sylvestre* for phytochemical using different solvent revealed that water and

methanol are the best among tested solvents. The results implies that using water and methanol for extraction of phytochemical material does not affect the quality of the compounds in the extract. This may likely be as a result of simple polanic effect of the solvents. Ethanol extraction may also be applicable but less effective for phytochemical compounds such as saponin and tannin. Petroleum ether, hexane and chloroform would not produce good extract for phytochemical analysis of *G. sylvestre* leaf materials. The present result was in agreement with the report of Ajaiyoba (2000) who demonstrated that water and methanol were most effective for phytochemical extraction of *Gynandropsis gynandra* and *Buchholzia coriacea*.

The two varieties of *G. sylvestre* showed variations in amount of phytochemical compounds evaluated as summarized (Table III). Quantitative study of carbohydrates, proteins and secondary metabolites that were present in the leaf in mg/g revealed that the quantity of saponin quantity was higher in MCL (5.6mg/g), while ZMGL contained 5.2mg/g of saponin. The differences in amount of phytochemical compounds present in the extract of the studied varieties could have arisen from different environmental and growth conditions of the plants since plants' chemical composition may be influenced by environmental factor. It was noted that the amount of phytochemical constituents of an extract depends on the chemical nature, quality, properties of constituents, the solvent employed, and the type of plant part and the method of extraction used (Trease and Evans 2005).

Table II. Showing presence or otherwise of phytochemical compounds in the leaves extract of *G. sylvestre* using different solvents.

Test	Reagents used	Water	Ethanol	Methanol	Pet. Ether	Hexane	Chloroform
Saponin	Foam formation	+	-	+	+	-	-
Tannin	2% FeCl ₃	+	-	+	-	-	-
Flavonoid	2% NaOH	-	+	+	-	-	-
Carbohydrate	Benedict's	+	+	+	-	-	+
Terpenoid	CHCl ₃ + H ₂ SO ₄	+	+	+	-	+	-
Cardiac glycoside	FeCl ₃ + glacial acetic acid+ H ₂ SO ₄	+	+	+	-	-	-
Alkaloid	Mayer's test	+	+	+	-	-	+

+ = positive (present); - = negative (not present)

Table III. Quantity of phytochemical compounds in the extracts of two lines of *G. sylvestre*

Compounds	MCL (mg/g)	ZMGL (mg/g)
Saponin	5.60	5.20
Tannin	0.10	0.40
Flavonoid	28.00	35.00
Carbohydrate	930.00	470.00
Alkaloid	120.00	109.00
Protein	0.30	0.27
Phenol	1.20	0.51
Free radical activity	17.00	35.00

MCL - Big-leafed variety; ZMGL-Small-leafed variety

Table IV. Showing physicochemical analysis of the two line of *G. sylvestre* leaf extracts.

Physiochemical property	MCL (gm/g)	ZMGL (gm/g)
Total ash	4.00	1.80
Acid insoluble ash	1.17	1.12
Water soluble extract	0.06	0.68
Ether soluble extract	0.66	0.45
pH value	5.90	5.00

MCL -Big-leafed variety; ZMGL- Small-leafed variety

Meanwhile, detailed and systematic pharmacognostical evaluation of the varieties may give valuable information for the future studies as opined by Kanetkar *et al.*, (2004).

The results for physicochemical parameters estimated are presented (Table IV). Estimation of ash value and extractive value from dried leaves of the two varieties showed that MCL contained higher proportion of ash (4.0mg/g) and ZMGL had low ash content of 1.8mg/g. The acid insoluble ash was also

higher in MCL (1.17mg/g) than ZMGL with 1.12mg/g of acid insoluble ash. The water soluble extracts of ZMGL was more soluble in water (0.68mg/g) than extracts of MCL (0.06mg/g). Physicochemical constituent such as ash value is significant in phytochemical evaluation as it gives an idea of the inorganic composition and other impurities present in the extracts. Extractive values could therefore be useful for the determination of purity of medicinal products as opined (Mukherjee, 2002).

Table V. Gymnemic acid Rf value estimation by TLC determination using different methods

No. of spot	Method 1		Method 2		Method 3		Method 4	
	MCL	ZMGL	MCL	ZMGL	MCL	ZMGL	MCL	ZMGL
1	0.17	0.34	0.07	0.31	0.64	0.81	0.63	0.65
2	0.24	0.42	0.19	0.38	0.67	0.82	0.53	0.49
3	0.34	0.48	0.32	0.44	0.91	0.89	0.46	0.34
4	0.53	0.61	0.46	0.58	0.87	0.41	0.38	0.39
5	0.19	0.83	0.90	0.77	0.30	1.9	0.36	0.42
6	0.31	---	0.15	0.79	---	---	---	---
7	0.42	---	0.26	---	---	---	---	---
8	---	---	0.38	---	---	---	---	---
9	---	---	0.90	---	---	---	---	---

MCL - Big-leafed variety; ZMGL- Small-leafed variety

Table VI. Comparative hypoglycemic effect of leaf extracts of two lines of *G. sylvestre* and standard drug glibenclamide. (Blood glucose level (mg/dL) at 30min. time interval)

		Control	STTD	ZMGL	MCL
0min		88.66±9.05	82.60±9.37	84.48±8.48	82.96±2.82
30min	Value	168.83±21.47	79.50±9.46*	74.50±13.43*	79.50±4.94*
	%R	-90.42	3.77	11.81	4.17
60min	Value	119.51±15.80	58.50±10.50*	60.00±4.24*	69.50±12.26*
	%R	29.21	26.41	19.46	12.5
90min	Value	119.50±18.94	50.98±12.86*	54.50±14.84*	56.01±9.89*
	%R	0.00	2.85	10.09	19.41
120min	Value	112.66±22.44	50.50±6.08*	51.02±5.65*	54.10±14.14*
	%R	5.73	0.94	6.38	3.41
150min	Value	104.01±23.37	46.33±9.73*	46.40±9.92*	47.05±9.89*
	%R	7.67	8.25	9.06	13.03

%R* = Percentage reduction in blood glucose level at 30min interval; * P < 0.01, as compared to control group are significantly different (One Way ANOVA followed by Tukey's Multiple Comparison Test); STTD-Standard drug glibenclamide; MCL-Big-leafed variety; ZMGL-Small-leafed variety

This may explain why most herbal pharmaceutical companies show preference for the small-leaf line (ZMGL) due to its higher phytochemical yield potentials.

Analysis of gymnemic acid extracted from the leaves of the two lines of *G. sylvestre* by evaluation of the Rf values using Thin Layer Chromatography (TLC) and employing four different methods showed different Rf values for the two varieties (Table V). Generally, nine spots were considered for the chromatography,

at spots 1-5, all the four methods produce different Rf values, but from spots 6-9, only method 2 had Rf values for ZMGL. Puratchimani and Jha, (2004) had earlier reported different Rf values for medicinal plant bioactive constituents extracted by different solvent methods. Other methods could not produce Rf values for most of the spots 6-9 (table V). Thin layer chromatography of the gymnemic acid indicated that some of the Rf values are similar when compared with the reference

Rf value and therefore implies that the purity of gymnemic acid obtained by such method is reliable.

Analysis of the blood samples from hyperglycemia induced whisker rats treated with extracts of *G. sylvestre* revealed similar with no significance difference to hypoglycemic activity of standard drug (glibenclamide) (Table VI). There is significance difference in treatment efficiency with respect to control however, no significance difference was found in the efficiency of the extracts in lowering the blood glucose level in the experimental animals and this concurred with the report of Ankit, *et al.*, (2010). Similar finding was earlier reported by Cretti *et al.* (2000) that plant extracts were efficient in lowering blood glucose. The hypoglycemia activities of gymnemic acid from the leaf extracts of the two varieties of *G. sylvestre* showed significant difference from untreated control and no significance difference with respect to treatment with a standard drug (glibenclamide). In fact after 30min of glucose challenge in the experimental animals, extracts from ZMGL was found to have significant effect in reducing the blood glucose. Hence, the leaf extract from *Gymnema sylvestre* have similar efficiency in lowering blood glucose of whisker rats used in this study and this is in conformity and corroborated earlier reports (Babu *et al.*, 2002; Nakamura *et al.*, 2010, Krishna *et al.*, 2012).

CONCLUSION

The use of plant extract to manage diseases and disorder has been long practiced by local folks mostly in the developing world. However, with economic regression and reported side effects of chemical drugs, the pendulum is gradually shifting toward the use of natural products which are locally available in abundance. This study evaluated the phytochemical characterization of compounds associated with *Gymnema sylvestre* using different solvents. It was discovered that water and methanol were the best solvents for extraction of phytochemical compounds from the plant. On the basis of the results of the present study, the gymnemic acid obtained from the plant extract proved efficient for reducing glucose level in experimental animals. When compared to standard drug glibenclamide, similar

hypoglycemic effects were achieved. However, considering the economic aspect, treatment and management of high level blood glucose is favoured by using extract of *G. sylvestre*.

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